Opti-TOF™ MALDI Plate System

Single-Use MALDI Plates for Voyager-DE™ Workstations and QSTAR® Systems with oMALDI™ Source

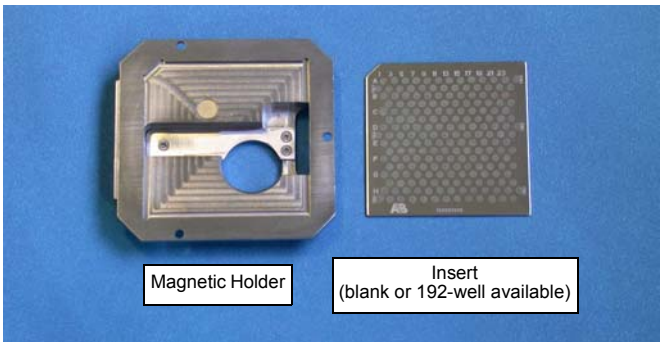
Applied Biosystems

Protocol

1 Overview

Applied Biosystems' Opti-TOF™ MALDI Plate System provides hydrophobically coated, single-use MALDI plates designed for high spot density, LC/MALDI, or robotic spotting applications.

The Opti-TOF MALDI Plate System includes a reusable holder and a single-use insert. The holder/insert design provides an inexpensive sample plate format that eliminates sample carryover and provides a convenient format for archiving sample plates for additional analysis.



Features

- Hydrophobically coated for uniform, reproducible, high-density spotting.
- 192-well inserts included in the kit, blank inserts available for purchase.
- Compatible with all Applied Biosystems MALDI platforms, allowing analysis of samples on different platforms without respotting (correct holder required).
- Additional inserts are spotted with β-galactosidase for instrument calibration (see Section 6 for peptide mass assignments).
- Inserts have serial numbers for easy sample tracking.
- On Voyager-DE Workstations, provides mass accuracy of:

  – 0.002% for runs with internal calibration
  – 0.008% for runs with OptiPlate calibration

- On QSTAR systems, provides mass accuracy of:

  – ±5 ppm for LC/MS runs with internal calibration
  – ±20 ppm for runs with external calibration

- Compatible with automated spotting devices including the Probot™ Micro Fraction Collector (available from Dionex Company), and the Applied Biosystems SymBiot® XVI Sample Workstation.

WARNING **CHEMICAL HAZARD**. Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). Always use adequate ventilation such as that provided by a fume hood. For additional safety guidelines, consult the MSDS.

2 Assembling a Plate

IMPORTANT! The holder includes a magnet. Keep the holder away from computers and computer media.

Wear powder-free gloves when handling inserts to avoid contaminating the hydrophobic surface.

1. Remove an insert from the shipping tray by gently bending the tray to pop a corner of an insert out of the tray, then grasping the insert with a gloved hand and removing.
2. Hold the holder in one hand.
3. In the other hand, hold an insert at an angle. Handle the insert by the edges to avoid touching the hydrophobic coating.
4. Align the notch in the insert with the notch in the holder, then drop the plate into the holder.
5. Ensure the face of the insert is flush with the face of the holder.

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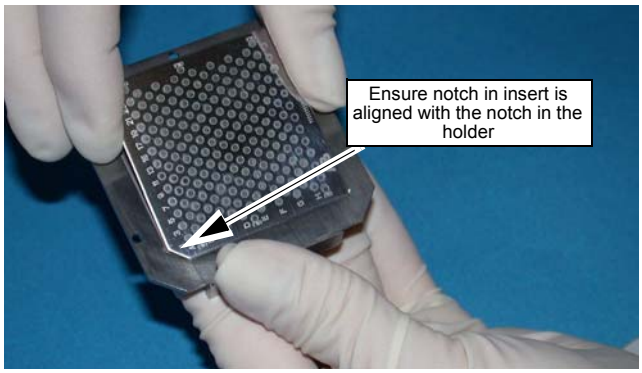

IMPORTANT! If the notch in the insert is not aligned with the notch in the holder, the plate will jam when you load it in the system.

6. If needed, adjust the insert so that the notched edge fits snugly into the notch in the holder by pushing your finger through the hole in the bottom of the holder, then moving the insert by pushing on the edges of the insert.

3 Spotting an Opti-TOF™ Plate

Wear powder-free gloves when handling inserts to avoid contaminating the hydrophobic surface.

IMPORTANT! Before spotting an Opti-TOF™ MALDI Plate System plate, use dry, compressed gas to blow off fibers that may be on the plate.

Suggested Matrix Formulation

WARNING **CHEMICAL HAZARD.** Read the MSDS before handling any chemical mentioned below, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
Alpha-cyano-4-hydroxycinnamic acid (CHCA) may cause eye, skin, and respiratory tract irritation.
Acetonitrile (ACN) is a flammable liquid and vapor. Exposure may cause eye and respiratory tract irritation and blood system damage.

Type of Run	Matrix Formulation
LC/MS	7 mg/mL alpha-cyano-4-hydroxycinnamic acid (CHCA) in a solution of acetonitrile and water (75:25 v/v) with 2% wt/wt addition of ammonium citrate: 1. Combine CHCA with acetonitrile:water (75:25 v/v) to yield 7 mg/mL concentration. 2. Combine 1 mL of matrix with 13 µL of a 10 mg/mL solution of ammonium citrate to yield a 2% wt/wt addition.
Other	Refer to the document appropriate for your system: • *Voyager™ Biospectrometry™ Workstaion User Guide* • *QSTAR Hardware Manual* **Note:** To run OptiPlate (Voyager Workstation), you need alpha-cyano-4-hydroxycinnamic acid (CHCA).

Internal Calibrant Concentration

For high mass accuracy in LC/MS runs, use an internal standard to calibrate each spot (for example, use the ACTH clip 18–39 (MH^+ = 2,465) at ~ 25 fmol).

Guidelines

- For acquisition, spot samples in any spot positions. Do not spot beyond the perimeter of the CAL spots on the outer edges of the insert.
- For QSTAR system plate calibration, spot four corner positions with CHCA matrix.
- For QSTAR 2-point MS/MS calibration, spot one position with a calibration standard such as Glu^1-Fibrinopeptide B and matrix.
- For Voyager Workstation OptiPlate calibration (5.1 series software), spot every other row and all perimeter positions with CHCA matrix and the calibration mixture specified in the *Voyager™ Biospectrometry™ Workstation User Guide*.
- Before handling the single-use sample plate, allow all spots to dry completely.
- Immediately before use, use dry, compressed gas to blow off any fibers on the plate.

Spotting Using a Probot™ Micro Fraction Collector

When spotting with a Probot Micro Fraction Collector:

- Install a 180 µm or 360 µm tip (available from Dionex).
- Install the Opti-TOF plate tray (available from Dionex) on the xyz platform by aligning the holes in the tray over the pins in the platform. Make sure the tray is flush with the platform by pressing down on the four corners of the tray. The Opti-TOF™ MALDI Plate System Plate tray is designed to hold the holder/insert assembly.
- Install the Opti-TOF plate holder/insert assembly in the tray. Position the holder/insert assembly with the notch to the left-rear corner of the tray.
- Align the tip with the registration marks in position A1. Depending on the tip size, align with the inner or outer etched ring in this position.
- Program the Probot:
  – Spotting pattern appropriate for your system.
  – Final flow rate (LC eluent and matrix) of 1 to 2 µL/min for a full plate of 576 spots. With fewer spots, you may be able to use a higher flow rate.
  – Distance Between Rows and Distance Between Columns set to **1.475** for a full plate of 576 spots.

Spotting Using a SymBiot® XVI Sample Workstation

For information on spotting an Opti-TOF™ MALDI Plate System plate with the SymBiot XVI workstation, refer to the *SymBiot® XVI Online Help*.

4 Analyzing a Spotted Opti-TOF™ Plate in a Voyager-DE™ Workstation

4.1 Obtaining the .PLT File

To obtain the 01-192+6-BB.PLT file to use with the 192-well insert:

1. Go to **www.appliedbiosystems.com**.
2. Click **Support**, then **Frequently Asked Questions**.
3. In the Search By field, select **Answer ID**.
4. In the Search text field, type **2172**, then click **Search**.
5. Detach the 01-192+6-BB.PLT file by right-clicking, then selecting **Save As**.
6. Copy the .PLT file to the C:\Voyager directory.

4.2 Analyzing

This section provides brief procedures. For detailed procedures, refer to the *Voyager™ Biospectrometry™ Workstation User Guide*.

1. Load the plate and select the 01-192+6-BB.PLT file.

   IMPORTANT! Make sure the notch in the insert is aligned with the notch in the holder. If the notches are not aligned, the plate will jam when you load it in the system.

2. Align the sample plate.
3. For optimum mass accuracy, run the OptiPlate software using the Opti-TOF™ MALDI Plate System plate (available with Voyager 5.1 series software). For information, refer to the *Voyager™ Biospectrometry™ Workstation User Guide*, and follow the recommendations for the 96 × 2-well plates.
4. Acquire unknown samples as described in the *Voyager™ Biospectrometry™ Workstation User Guide.*

5 Analyzing a Spotted Opti-TOF™ Plate in a QSTAR® System

This section provides brief procedures. For detailed procedures, refer to the *oMALDI™ Server User's Manual* or the *Analyst® QS Software Getting Started Manual*.

Perform the plate calibration as needed using the oMALDI software:

1. Load the plate.

   IMPORTANT! Make sure the notch in the insert is aligned with the notch in the holder. If the notches are not aligned, the plate will jam when you load it in the system.

2. Create a plate type if needed.
3. Select the plate type.
4. Perform a plate calibration (aligns the plate).

Perform a mass calibration as needed:

1. In the oMALDI software, select the plate calibration file.
2. In the Analyst QS software, acquire data from the calibration spot in manual or batch mode.
3. Perform a 2-point MS/MS calibration on a position spotted with a calibration standard (for example, if you use Glu^1-Fibrinopeptide B, calibrate with the m/z 175 and 1,570.67 peaks). The MS/MS calibration is valid for MS and MS/MS modes.
4. Acquire unknown samples with the Analyst software as described in the documentation provided with your system.

6 β-Galactosidase Peptide Mass Assignments

Fragment Number	Peptide Fragment	Residues (Start–End)	$(M+H)^+$ Monoisotopic, Calculated (Da)
T10	WVGYGQDSR	159–167	1,067.4910
T77	GDFQFNISR	954–962	1,083.5223
T64	IDPNAWVER	802–810	1,099.5536
T76	ELNYGPHQWR	944–953	1,299.6234
T11	LPSEFDLSAFLR	168–179	1,394.7320
T3	DWENPGVTQLNR	16–27	1,428.6872
T52	LSGQTIEVTSEYLFR	632–646	1,742.8965
T72	VNWLGLGPQENYPDR	896–910	1,757.8611
T14	WSDGSYLEDQDMWR	192–205	1,787.7335
T48	YDENGNPWSAYGGDFG DTPNDR	579–600	2,446.9812
T70	IDGSGQMAITVDVEVASD TPHPAR	859–882	2,466.1935
T74	WDLPLSDMYTPYVFPSE NGLR	919–939	2,516.1808
T20	VTVSLWQGETQVASGTA PFGGEIIDER	257–283	2,847.4165

7 Troubleshooting

Symptom	Possible Cause	Action
Voyager Poor mass accuracy in Delayed Extraction mode (external calibration only)	Mass Accuracy Optimization option not enabled when plate was loaded.	Enable Mass Accuracy Optimization. For information, see the *Voyager™ Biospectrometry™ Workstaion User Guide*.
Spots are running together during spotting	Hydrophobic surface was compromised (for example, the plate was washed).	Use a new insert.
Finger prints on unspotted insert.	Insert was handled without powder-free gloves.	Rinse with 100% isopropanol, then dry and buff with a lint-free lab tissue.

WARNING **CHEMICAL HAZARD.** Isopropanol is a flammable liquid and vapor. Exposure may cause eye, skin, and upper respiratory tract irritation. Prolonged or repeated contact may dry skin and cause irritation. Exposure may cause central nervous system effects such as drowsiness, dizziness, and headache. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

8 Storing and Archiving Inserts

Store spotted or unspotted inserts at room temperature in the original packing with the desiccant provided. Protect from light and dust, and keep dry.

In a test study using an insert spotted with a trypsin digest of β-galactosidase, no decrease in sensitivity was observed after 68 days of storage. Stability of your samples may differ based on composition.

9 Disposal of Inserts

Dispose spotted analyzed inserts according to local, state, and federal regulations.

10 Cleaning the Holder

Clean the holder as needed. Use acetone or 50% acetonitrile:water. Dry the holder completely after washing to prevent corrosion of the magnet.

11 Technical Support

Applied Biosystems is committed to meeting the needs of your research through enabling technologies such as the Opti-TOF™ MALDI Plate System. Our dedicated support staff is available to answer questions about using this product to its fullest potential.

Contacting Technical Support in North America

To contact technical support:

- By telephone: Dial **1**.**800**.**899**.**5858**
- By fax: Dial **1**.**508**.**383**.**7855**

12 Ordering Information

To place an order from the U.S. or Canada, dial **1**.**800**.**327.3002**, then follow the voice instructions.

Description	Quantity	Part Number
Opti-TOF™ 192-Well Plate Kit for QSTAR® and Voyager™ Systems (includes QSTAR/Voyager system holder, 5 192-well inserts, five 192-well inserts spotted with β-galactosidase)	1 kit	4347686
Holder, Opti-TOF, 4700 System	1	4347690
Holder, Opti-TOF, QSTAR and Voyager -DE™ Systems	1	4347689
Insert, Opti-TOF, blank	25/pk	4347680
Insert, Opti-TOF, blank	100/pk	4347683
Insert, Opti-TOF, 192-well	25/pk	4347682
Insert, Opti-TOF, 192-well	100/pk	4347681
Insert, Opti-TOF, β-galactosidase pre-spotted, 192-well	5/pk	4347687
Adapter tray for Probot™ Micro Fraction Collector	1	Contact Dionex

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U.S. patents pending.

The 4700 Proteomics Analyzer and its use are covered by one or more of the following U.S. Patents: 5,625,184; 5,627,369; 5,760,393; 6,002,127; 6,057,543; 6,281,493, and 6,348,688. Additional U.S. and foreign patents are pending.

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Headquarters
850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

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